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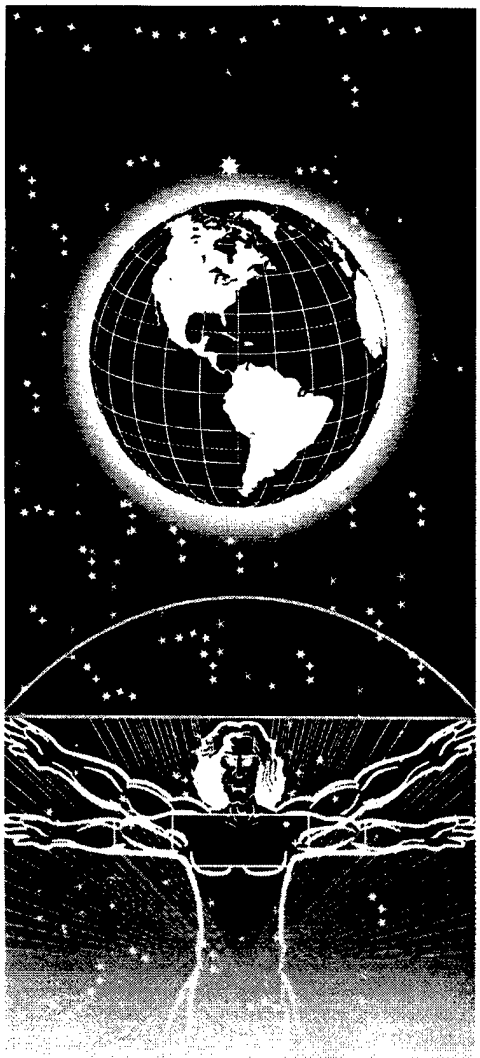

UNITED STATES AIR FORCE ARMSTRONG LABORATORY

Acute, Subchronic and Reproductive Toxicity of Quadricyclane Vapor on Sprague-Dawley Rats

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TECHNICAL REVIEW AND APPROVAL

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The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals* prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

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The U.S. Air Force is currently developing High Energy Density Matter (HEDM) for use in advanced rocket propellants to improve their performance. An HEDM of immediate interest is Quadricyclane. Assessments were performed to determine the inhalation toxicity of Quadricyclane vapor. An acute 4-h exposure of male Sprague-Dawley rats to 5mg Quadricyclane/L resulted in complete mortality. Additional acute exposures to Quadricyclane determined an LC_{50} for male rats of 0.78mg/L. Subchronic inhalation exposures on groups of 10 male and 10 female Sprague-Dawley rats at concentrations of 0.0, 0.025, 0.075, and 0.25mg/L resulted in no mortality. Body weights of Quadricyclane-exposed rats were significantly less than those of controls after 10 exposures. No exposure-related gross lesions were noted at necropsy. A 90-day general toxicity/reproductive screen using concentrations of 0.0, 0.1, 0.025, and 0.01mg/L produced no significant exposure-related differences in the reproductive or litter parameters measured. The only lesion noted at necropsy was minimal pulmonary inflammation in the lungs of the mid- and high-concentration male rats. Significant treatment-related increases in neurotransmitters dopamine and 5-hydroxytryptamine were detected in all quadricyclane-exposed rats. The low-concentration level of 0.01mg Quadricyclane/L probably represents a NOEL based upon the female rat data, and the lack of consistent dose-response data for male rats.

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PREFACE

This is one of a series of technical reports describing results of experimental laboratory programs conducted at the Toxic Hazards Research, ManTech Geo-Centers Joint Venture. This document serves as a final report on the acute, subchronic, and reproductive toxicity of quadricyclane vapor on Sprague-Dawley rats. The research described in this report began in March 1995 and was completed in June 1996 under Department of Defense Contract Nos. F33615-90-C-0532 and F41624-96-C-9010. Lt Col Terry Childress served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Darol E. Dodd, Ph.D., served as Program Manager for the Toxic Hazards Research.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

The authors gratefully acknowledge David H. Ellis, Richard J. Godfrey, Jerry W. Nicholson, and Margaret Parish for their excellent technical assistance. Also acknowledged is Carlyle D. Flemming for statistical analysis of the data.

ABBREVIATIONS

ALT	Alanine aminotransaminase
AST	Aspartate aminotransaminase
BUN	Blood Urea Nitrogen
°C	Degrees Celcius
CO_2	Carbon Dioxide
DA	Dopamine
DHBA	3,4-Dihydroxybenzylamine hydrobromide
Dopac	3,4-Dihydroxyphentylacetic acid
E	Epinephrine
°F	Degrees Fahrenheit
FL	Femtoliter
GD	Gestation Day
g/dL	Grams per Deciliter
g/kg	Grams per Kilogram
g/ml	Grams per Milliliter
h	Hour
HCT	Hematocrit
HEDM	High Energy Density Matter
HGB	Hemoglobin
HPLC	High Performance Liquid Chromatography
5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-Hydroxytryptamine
HVA	Homovanillic acid
IU/L	International Units per Liter
L	Liter
LC_{50}	Median Lethal Concentration
LD	Lactation Day
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
mg	Milligram
mg/dL	Milligram per Deciliter
mg/kg	Milligram/Kilogram
mg/L	Milligram per Liter
min	Minute
mL	Milliliter
mM	Millimoles
mmHg	Millimeters of Mercury
mmol/dL	Millimolar per Deciliter
mmol/L	Millimolar per Liter
N	Number
nA	NanoAmpers
NE	Norepinephrine
ng	Nanogram
p	Probability
PD	Postpartum Day
pg	Picogram

ppm	Parts per Million
psi	Pounds per Square Inch
RBC	Red Blood Cell
RP	Rocket Propellants
SEM	Standard Error of the Mean
V	Volts
WBC	White Blood Cell
wt	Weight
w/v	Weight/Volume
µg	Microgram
µL	Microliter
µm	Micrometer

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SECTION I

INTRODUCTION

The Air Force is currently developing High Energy Density Matter (HEDM) for use as advanced rocket propellants (RP). The most near-term development effort is that of the strained-ring hydrocarbons. These compounds will be added to propellant RP-1 (kerosene) to improve performance. An HEDM of immediate interest is quadricyclane (CAS 278-06-9). Present plans are for producing a rocket fuel mixture of 70% quadricyclane and 30% RP-1.

Very little toxicologic data are available for quadricyclane. The acute oral and dermal toxicity of the compound and a mixture of quadricyclane in Kerosene was reported by Kinkead et al. (1993). An oral dose of 3.5 g/kg body weight of the neat compound to male F-344 rats resulted in 100% mortality within 24 hours. An oral dose (1.7 g/kg) of the 70/30 (quadricyclane/RP-1) mixture produced prostration in all rats through 24 hours posttreatment, but no mortality. Quadricyclane did not cause mortality when applied dermally to rabbits at a dose of 2 g/kg.

Quadricyclane will undergo metal ion catalyzed rearrangement to norbornadiene with the release of heat. RJ-5 is a mixture of dimers of norbornadiene and is a component of the jet fuel JP-9. The acute oral toxicity of RJ-5 has been reported by Haun et al. (1978). A peroral dose of 4 g RJ-5/kg body weight administered in corn oil to three rats was not lethal. Haun also reported that rats survived an atmosphere of essentially saturated vapor of RJ-5 for 6 hours. However, RJ-5 has a low vapor pressure (0.025 mmHg). A six-month inhalation study of 0.15 mg/L RJ-5 resulted in pulmonary irritation to dogs and monkeys (Haun et al., 1978). The vapor pressure of quadricyclane is much higher than that of RJ-5 and may pose a much greater hazard by the inhalation route of exposure.

The approach for determining the inhalation toxicity of quadricyclane was divided into three areas:

1) Acute, (4-hour) inhalation exposures to identify signs of toxic stress. This information is necessary to establish limits for emergency conditions resulting from spills and accidents

2) Subchronic exposures (repeated over 2 weeks) to determine potential cumulative toxic effects and for setting exposure concentrations in the definitive 90-day study

3) Long-term (90-day) general toxicity/reproduction screen exposures to provide information for normal daily operations or threshold limit values and on possible gonadal effects.

These data can be applied by Air Force medical and safety authorities in specifying operational procedures, protection equipment and control measures.

SECTION 2

EXPERIMENTAL APPROACH

Animals

Male and female Sprague-Dawley-derived outbred albino rats [Crl:CD®(BR)] were purchased from Charles River Breeding Laboratories, Raleigh, NC. Rats used in the acute and subchronic studies were 7 weeks of age and those in the reproductive screen were 9 weeks of age upon receipt. All rats were identified by tail tattoo and were acclimatized two weeks prior to use. During the acclimation period, quality control procedures were performed on selected rats as described in Kinkead et al. (1991).

Rats in the subchronic and reproductive screen studies were assigned to groups by means of a computer-generated randomization. The randomization was stratified by body weight such that the mean body weights of all groups were homogeneous by statistical analysis at study initiation. Water from a reverse-osmosis system and Purina Formulab #5002 feed were available *ad libitum*. Animal rooms were maintained on a 12-h light/dark cycle (flourescent light) and targeted at a temperature of 23 ± 2 °C and a relative humidity of 55 ± 15%.

Test Agent

The quadricyclane test compound was purchased from EniChem America, Inc., Houston, TX. Pertinent physical and chemical properties are listed below:

CAS number:	278-06-8
Appearance:	Colorless liquid
Empirical formula:	C_7H_8
Formula weight:	92.14
Boiling point:	108 °C
Specific gravity:	0.919 g/mL
Purity:	>95%

Test Agent Quality Control

The purity of the quadricyclane was determined by Gas Chromatography-Mass Spectrometry. The samples were analyzed using the following instrumentation and operating parameters:

Hewlett-Packard* 5971A Series Mass Selective Detector
Hewlett Packard* 5890A Series II Gas Chromatograph
Hewlett-Packard* Vectra 486/66U Data System
Hewlett-Packard* 7673 Autosampler/6C Injector
Hewlett-Packard* 18596B Sample Tray
Analytical column: DB624, 30m x 0.25 mm ID, 1.4µm film thickness.
6C program: 45 °C - 10.0 min/15 °C per min/150 °C - 5.0 min.

*Hewlett-Packard Co., Palo Alto, CA.

Samples taken from each of the two containers received were analyzed for purity. Solutions were prepared at concentrations of approximately 5 mg quadricyclane/mL methanol. The results for these samples indicate a purity of 94%, with two major contaminants that appear to be related compounds with mass spectra similar to quadricyclane. Library searches yielded matches with tetracycloheptane, bicycloheptane, cycloheptatriene, and spiroheptadiene. The greatest contaminant was bicycloheptane at a level of approximately 3.6 %.

Generation and Analysis of Exposure Atmospheres

Quadricyclane vapor was generated by continuously metering known amounts via syringe pump (Sage model 355, Sage Instrument Division, Cambridge, MA) into the chamber input air stream. The carrier air for the quadricyclane vapor was maintained a flow rate between 9 and 10 L/min and entered the chamber input air stream in a counter current flow to aid in mixing. The atmospheres generated for the 4-hour acute inhalation study were conducted in 30-L bell jar chambers were analyzed throughout the exposure (every 6 to 7 minutes) using a Varian 3400 gas chromatograph (Varian Associates, Palo Alto, CA). The chamber atmospheres for the 2-week subchronic study were continuously analyzed using Miran 1A infrared analyzers (Foxboro Analytical, South Norwalk, CT) equipped with 20-M path gas cells.

The low- and mid-concentration chamber atmospheres of the 90-day reproductive screen were analyzed continuously using Beckman 400 total hydrocarbon analyzers (Beckman Instruments, Inc., Fullerton, CA). The high-concentration chamber was analyzed using a Miran 1A equipped with 20-M path gas cells.

Acute Exposures

Groups of 6 male rats, approximately 10 weeks of age, were placed in a 30-L bell jar chamber and exposed once for 4 hours. The initial exposure was at the limit test concentration of 5 mg/L. Dilutions of this concentration followed to achieve a NOAEL concentration. All rats were maintained for a 14-day postexposure observation period. Records were maintained for body weights (Days 0, 7, and 14 postexposure), signs of toxic stress, and mortality. Gross pathology was performed at necropsy. Histopathology was performed on lungs and any lesions of animals that died on study.

Subchronic Inhalation Exposures/Repeated Two-Week

Exposure groups consisted of 10 male and 10 female rats approximately 9 weeks of age. The rats were exposed for 6 hours daily for 2 weeks, excluding weekends (10 exposures over a 2-week period). Because exposures of female rats were initiated one day after the male rats, the total number of exposure days analyzed was 11; however, each group received only 10 exposures. Exposure concentrations were 0.0, 0.025, 0.075, and 0.25 mg quadricyclane/L. The rats were singly housed in wire-bottom cages during exposure and in plastic shoe-box cages with bedding during nonexposure periods.

Records were maintained for body weights (Days 0 ,7, and 14), and a clinical examination was performed on each exposure day. At necropsy, blood samples were taken via the vena cava for standard hematology and clinical pathology analyses. Gross pathology was performed on all animals. Wet tissue weights were determined on brain, kidneys, liver, lungs, spleen, and thymus.

General Toxicity/Reproductive Toxicity Screen

Exposure Regimen

Twelve male and twelve female rats per group were placed in 690-L inhalation chambers and exposed for 6 h daily to air only, 0.01, 0.025, or 0.1 mg quadricyclane/L. Each chamber held four exposure cages. Each of these cages had eight separate sections to contain rats during exposure. The animals were housed individually and randomly assigned to specific exposure cage locations. The exposure cages were rotated clockwise (moving one position) within the inhalation chambers each day. Animals were exposed 5 days/week for 2 weeks prior to mating. Males and females were exposed 7 days/week during mating, gestation, and lactation. Following weaning of the last litter, the rats returned to a 5 day/week exposure regimen. Dams were excluded from exposure Gestation Day (GD) 21 through Postpartum Day (PD) 4. Pups were not exposed at any time during the study.

One male and one female were cohabited, selected randomly from within their respective exposure groups, starting on Study Day 14. The pairs remained cohabited during nonexposure hours for up to 14 days until either a copulation plug was observed or sperm were present in the vaginal wash. The day a copulation plug was present or sperm were noted in the vaginal wash was defined as GD 0.

Rats were observed twice daily for signs of toxic stress. Male rat body weights were measured weekly during the study. Female body weights were measured in the same manner until confirmation of mating. During gestation, females were weighed on GD 0, 7, 14, and 20. Dams producing litters were weighed on PD 0, 4, 7, 14, and 21, then weekly thereafter. All pups were counted and sexed on PD 0. Live pups were weighed 1, 4, 7, 14, and 21 days after birth. Standardization of litter sizes, 4 per sex when possible, occurred on PD 4. All pups were examined for external abnormalities during the lactation period and received a gross examination when necropsied at weaning (PD 21).

Clinical Measurements

At necropsy, blood samples were taken via the vena cava from fasted parental animals for hematology and clinical chemistry assays (Table 1). Erythrocytes were enumerated on a Coulter counter (Coulter Electronics, Hialeah, FL) and sera for clinical chemistry evaluations were assayed on an Ektachem 700XR (Eastman Kodak, Rochester, NY). Selected hematological parameters and absolute leukocyte differentials were determined according to established procedures. Sera were processed according to the procedures in the Ektachem Operations manual.

Table 1. Serum Chemistry and Whole Blood Assessments from Control and Quadricyclane-Treated Sprague-Dawley Rats

Serum		Whole Blood
Albumin	Magnesium	Hematocrit
Alkaline phosphatase	Phosphorus	Hemoglobin
Alanine aminotransaminase	Potassium	Red blood cell count
Aspartate aminotransaminase	Sodium	Total and differential leukocyte count
Bilirubin	Total protein	
Calcium	Triglycerides	Platelet count
Chloride	Urea nitrogen	
Cholesterol		
Creatinine		
Glucose		

Evaluations at Necropsy

Brain, liver, kidneys, spleen, thymus, testes, and epididymides were weighed at necropsy. Bouin's fixative was used to fix the testes and epididymides. The pituitary, spleen, liver, lungs, kidneys, bone with bone marrow, and reproductive organs were removed from parental animals of both sexes and fixed in 10% buffered formalin solution. After routine processing, the tissues were embedded in paraffin and stained with hematoxylin and eosin for histopathologic examination.

Neurotransmitter Analysis

Neurotransmitter analysis was performed on 6 male and 6 female rats per group from the study. At final sacrifice, the brains from these animals were surgically removed at sacrifice, weighed whole, and then nine regions of each brain were dissected, frozen on dry ice, and stored at -70 °C until analysis. The nine brain regions analyzed were the brain stem, frontal cortex, cerebral cortex, caudate nucleus, septum, hypothalamus, thalamus, hippocampus, and cerebellum. Each region was analyzed for four neurotransmitters, norepinephrine (NE), epinephrine (E), dopamine (DA), and 5-hydroxytryptamine (5-HT). Metabolites of DA, homovanillic acid (HVA), and 3,4-dihydroxyphenylacetic acid (Dopac), and a metabolite of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), were also analyzed.

Analysis of the neurotransmitters was performed following the methods described in Kim et al., 1987. The frozen tissue samples were thawed and homogenizing each sample for 30 seconds in 0.17 M perchloric acid (90 mg tissue/1.0 mL perchloric acid) containing 125 ng of 3,4-dihydroxybenzylamine hydrobromide (DHBA), used as an internal standard. A Polytron homogenizer (GLAS-COL, Terre Haute, IN) was used for homogenizing the samples. Homogenates were centrifuged at 31500 g for 30 minutes at 4 °C. The supernatants were separated and immediately analyzed by injecting 20 µL of the supernatant in a high performance liquid chromatograph using an autosampler.

High performance liquid chromatography (HPLC) determinations were performed with a Dionex Model DX-300 isocratic liquid chromatograph (Dionex Corporation, Sunnyvale, CA) coupled with a pulse electrochemical detector (PED-2). An advanced gradient pump (Dionex, APG Standard size) was also used. A glass-carbon working electrode (Dionex Corp.) was maintained between 0.5 and 1.0 nA depending upon the concentration of the neurotransmitters. Separation by isocratic elution was performed on a C_{18} reverse phase column preceded by a guard column (Guard-Pak, C_{18} Waters Association, Milford, MA).

The mobile phase was 15% (w/v) methanol in a solution (pH 4.2) of 32 mM citric acid, 12.5 mM disodium hydrogen orthophosphate, 0.5 mM octyl sodium sulfate, and 0.05 mM disodiumethylenediaminetetraacetic acid. The mobile phase was filtered through a 0.45-µm filter (Millipore, Bedford, MA) and then degassed under vacuum before use. A flow rate of 1.2 mL/min at 2200 psi under ambient temperature conditions was used.

Known amounts of each of the four neurotransmitters and the metabolites Dopac, HVA, and 5-HIAA were injected into the HPLC system in the range 0.2 to 20.0 ng. DHBA (2.5 ng) was used as an internal standard. All compounds were easily oxidized at 0.8 V versus a Ag/AgCl reference electrode (Dionex Corporation, Sunnyvale, CA). Each of these compounds gave a linear response in the 0.2 to 20.0 ng range.

Statistical Analysis

LC_{50} values were calculated using the method of Weil (1952). Maternal body weights, pup weights, organ weights, organ-to-body weight ratios, serum chemistry, and hematology were analyzed for statistical significance using a one-factorial analysis of variance with Bonferroni multiple comparisons (Rosner, 1990). A one-factorial repeated measures analysis of variance with Bonferroni multiple comparisons was used for paternal body weights (Barcikowski, 1983). Neurotransmitters and their metabolites were quantitated using linear regressions of their standards. Mating indices and histopathologic results were analyzed by a Chi-square test of proportions applied to the incidence data (Rosner, 1990). Tissue lesion severity data were analyzed using the Kruskal-Wallis analysis of variance (Rosner, 1990).

SECTION 3

RESULTS

Acute Exposures

Inhalation exposures to the limit test concentration (5mg/L) of vaporized quadricyclane resulted in 100% mortality. Additional 4-h inhalation exposures were performed to determine an LC_{50} value (Table 2). Exposure concentrations of 2.0, 0.5, 0.1 and 0.05 mg/L were selected to produce mortality rates between 0 and 100%.

During the exposures, the test animals exposed to the four highest concentrations of Quadricyclane were mostly inactive and did not respond to tapping on the chamber walls. Rats exposed at 0.05 mg/L remained active during exposure and showed a normal startle response to tapping on the chamber walls. Results of immediate postexposure observation included excessive salivation in rats exposed at the three highest levels; the 5.0 mg/L animals also demonstrated hyperesthesia (hypersensitivity to touch). Following death, all rats from the 5.0 mg/L exposure exhibited perioral and perinasal wetness. This was not observed in animals that died at lower concentration levels.

Gross pathology of animals that died from exposure revealed congested, bright-red colored lungs. Microscopic findings in these subjects included scattered aggregates of intravascular neutrophils, present in the lung, liver, and heart. Similar findings were present multifocally in the liver sinusoids of these animals. All rats that survived the 14-day observation period had normal lungs.

All deaths occurred within 12 hours of exposure. Animals that survived the initial 12 hours postexposure appeared normal and survived through the 14-day observation period.

Survivors of the quadricyclane exposures that produced lethality in some rats gained weight during the second week. Rats from the exposure groups producing no lethality gained weight throughout the 14-day observation period and no signs of toxicity were observed (Table 3). The LC_{50} value (95% confidence limit) for male rats was 0.78 (0.29-1.65) mg/L.

Table 2. Quadricyclane Concentration and Mortality Ratios for Acute 4-h Inhalation Exposures

Exposure Concentration (mg/L)		Mortality Ratio
Nominal	Measured[a]	(Deaths/Group size)
5.0	5.01 ± 0.02	6/6
2.0	2.05 ± 0.06	5/6
0.5	0.53 ± 0.06	2/6
0.1	0.10 ±<0.01	0/6
0.05	0.052 ± 0.002	0/6

[a]Mean ± SEM.

Table 3. Body Weights[a] of Surviving Male Rats After Acute 4-h Inhalation Exposure to Quadricyclane

Animal No.	Day 0	Day 7	Day 14
	2.0 mg/L		
11	322.4	335.1	370.8
	0.5 mg/L		
13	403.8	402.4	445.1
14	382.3	403.6	442.5
15	369.3	387.2	433.6
16	422.1	401.0	456.9
	0.1 mg/L		
19	396.6	415.2	446.6
20	365.1	394.7	426.8
21	399.4	425.9	449.5
22	411.7	428.0	438.9
23	426.0	447.3	491.4
24	327.9	334.1	362.3
	0.05 mg/L		
25	397.5	431.7	445.7
26	392.0	427.9	448.3
27	378.8	404.9	424.1
28	410.4	446.9	464.3
29	354.5	370.8	383.1
30	434.7	490.8	508.5

[a]Weight in grams.

Subchronic Exposures

During the two-week exposure period, daily mean concentrations of quadricyclane were maintained close to the desired concentration (Table 4). There were no mortalities during the study. There were numerous white "plugs" found in the excreta pans of the high-concentration male rats at the conclusion of the daily exposures. A few were found in the excreta pans of the other treated and control male rats. The characteristics of these crystals were consistent with calcium oxylate which is normally found in rat urine. Examination of plug material which was fixed in 10% neutral-buffered formalin, sectioned, and stained with H&E revealed a smooth, non-crystaline consistency which was identical to that of reflux proteinaceous coagulum which is normally found in the bladders of rats. The high-concentration animals, both male and females, became more aggressive with continued exposure. The rats displayed hyperesthesia and were aggressive towards each other.

Mean body weights of the quadricyclane-exposed rats were significantly lower than controls at 7 and 14 days. At 14 days, the exposed male rat groups were 14, 9.5, and 8% less ($p<0.01$) than controls while the female groups were 14, 15, and 11% less ($p<0.01$) than controls in the high-, mid-, and low-concentration levels, respectively. Alanine aminotransaminase ($p<0.01$), creatinine ($p<0.05$), and calcium ($p<0.01$) values were all decreased in the high-concentration male rats. Serum chemistry parameters in the exposed female rats were not different from controls. Hematology values were unaffected in both sexes.

Absolute liver weights were decreased in high-concentration male rats, and absolute lung weights were decreased in mid-concentration male rats (Table 5). Numerous differences in absolute organ weights were noted in all exposed quadricyclane female rats (Table 6). However, when these weights are corrected for body weights (ratio), the only differences noted are relative brain weights in all quadricyclane exposed female rats and the high-concentration male rats. No exposure-related gross lesions were noted at necropsy.

Table 4. Analysis of Quadricyclane Concentrations Inhaled by Male and Female Rats for Two Weeks (N=11)

Target Concentration, mg/L	0.250	0.075	0.025
Mean Measured Concentration, mg/L	0.252	0.075	0.025
Standard Error	0.001	0.001	0.001
Lowest Daily Average, mg/L	0.244	0.073	0.019
Highest Daily Average, mg/L	0.256	0.078	0.026

Table 5. Absolute and Relative Organ Weights[a] of Male Rats Following Treatment with Quadricyclane Vapor for Two Weeks

	Control	0.025 mg/L	0.075 mg/L	0.25 mg/L
Body wt.	354 ± 10.0	329 ± 7.5[d]	323 ± 6.1[c]	302 ± 6.8[c]
Brain	1.96 ± 0.04	1.94 ± 0.02	1.95 ± 0.04	1.91 ± 0.06
Ratio[b]	0.56 ± 0.01	0.59 ± 0.02	0.61 ± 0.01	0.63 ± 0.02[c]
Liver	10.97 ± 0.54	9.88 ± 0.29	10.00 ± 0.37	9.15 ± 0.23[d]
Ratio	3.08 ± 0.07	3.00 ± 0.06	3.10 ± 0.09	3.03 ± 0.04
Kidneys	2.70 ± 0.11	2.64 ± 0.09	2.61 ± 0.11	2.44 ± 0.08
Ratio	0.76 ± 0.01	0.80 ± 0.01	0.81 ± 0.03	0.81 ± 0.01
Spleen	0.71 ± 0.04	0.69 ± 0.06	0.69 ± 0.04	0.60 ± 0.03
Ratio	0.20 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.20 ± 0.01
Thymus	0.61 ± 0.05	0.53 ± 0.03	0.56 ± 0.05	0.49 ± 0.03
Ratio	0.17 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.16 ± 0.01
Lungs	1.88 ± 0.08	1.71 ± 0.05	1.61 ± 0.02[d]	1.77 ± 0.06
Ratio	0.53 ± 0.02	0.52 ± 0.01	0.50 ± 0.01	0.58 ± 0.01

[a]Mean ± SEM, N=10.
[b]Organ weight/body weight x 100.
[c]Significantly different from Control at $p<0.01$.
[d]Significantly different from Control at $p<0.05$.

Table 6. Absolute and Relative Organ Weights[a] of Female Rats Following Treatment with Quadricyclane Vapor for Two Weeks

	Control	0.025 mg/L	0.075 mg/L	0.25 mg/L
Body wt.	265 ± 5.4	237 ± 4.6[c]	220 ± 3.2[c]	225 ± 3.0[c]
Brain	1.88 ± 0.02	1.86 ± 0.01	1.88 ± 0.04	1.90 ± 0.03
Ratio[b]	0.71 ± 0.02	0.79 ± 0.02[c]	0.86 ± 0.02[c]	0.84 ± 0.01[c]
Liver	10.06 ± 0.34	8.87 ± 0.34	7.49 ± 0.21[c]	8.07 ± 0.15[c]
Ratio	3.79 ± 0.07	3.74 ± 0.12	3.40 ± 0.07	3.59 ± 0.05
Kidneys	2.04 ± 0.04	1.82 ± 0.06[d]	1.79 ± 0.03[c]	1.78 ± 0.06[c]
Ratio	0.77 ± 0.01	0.77 ± 0.02	0.81 ± 0.02	0.79 ± 0.03
Spleen	0.58 ± 0.02	0.49 ± 0.02[d]	0.51 ± 0.00	0.50 ± 0.02[d]
Ratio	0.22 ± 0.01	0.21 ± 0.01	0.23 ± 0.00	0.22 ± 0.01
Thymus	0.58 ± 0.04	0.41 ± 0.03[d]	0.39 ± 0.02[c]	0.44 ± 0.02[d]
Ratio	0.22 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.20 ± 0.01
Lungs	1.72 ± 0.14	1.42 ± 0.03[c]	1.34 ± 0.04[c]	1.42 ± 0.05[c]
Ratio	0.64 ± 0.04	0.60 ± 0.01	0.61 ± 0.01	0.63 ± 0.02

[a]Mean ± SEM, N = 10.
[b]Organ weight/body weight x 100.
[c]Significantly different from Control at $p<0.01$.
[d]Significantly different from Control at $p<0.05$.

General Toxicity/Reproductive Screen

During the 90-day inhalation exposure period, daily mean concentrations of quadricyclane were maintained near of the target concentrations (Table 7). There were a total of 84 exposures during the study.One male control rat had severe malocclusion and was eventually sacrificed 76 days into the study. The rat developed an oral infection and became moribund, necessitating euthanasia. Because of the resultant eating problems, this animal was always underweight and as a result was excluded from the body weight tables of the male control rats. No exposure-related mortalities occurred in any of the parental rats during the study. The high-concentration animals began to demonstrate hyperesthesia during the second week of exposure and became more aggressive with continued exposure. These animals would attempt to aggressively attack each other when exposure cages were opened for unloading the animals after the daily exposures. They also attempted to bite the technicians removing them from the exposure cages. Upon being held, these animals would fight to escape and squeal until they were placed into their home cages. The mid-concentration female rats became aggressive and sensitive to touch during the gestation period. The low-concentration animals did not demonstrate signs of hyperesthesia or aggressiveness. Numerous white "plugs" were found in the excreta pans of all male rat groups including control. These plugs were determined to be like those found during the subchronic exposure and consisted of proteinaceous coagulum which is normally found in the bladders of rats.

A concentration-related effect was noted in the mean body weights of both male and female rats (Figures 1 and 2). Mean body weights of the high-concentration male rats were significantly less than controls after one week of exposure and continued in that manner throughout the study (Appendix A). The mid-concentration male rats showed a decrease beginning at 34 days and the low-concentration males at 41 days which also continued throughout the study. Mean body weights of the high-concentration and mid-concentration female rats were significantly less than controls beginning on Day 13 (Appendix B). Low-concentration female rats' mean body weights were not different from control values during the study.

No exposure-related diffferences were noted in male or female hematologic parameters at necropsy (Tables 8 and 9). A number of serum chemistry values were statistically different than control values in the male rats (Table 10). However, the only differences that appear concentration and/or exposure-related are a decrease in potassium and creatinine and an increase in sodium. Potassium was also decreased in female rats at the high-concentration levels (Table 11). Total protein was decreased in the high- and mid-concentration female rats.

Absolute liver weights of the high-concentration male and female rats were statistically less ($p<0.01$) than controls (Table 12). Relative testis weights were increased in all quadricyclane-exposed male rat groups. The decrease in liver weights appears to be a result of the difference in mean body weights of the groups. The liver to body weight ratios indicate no differences.Relative testes weights did not change proportionally to mean body weight.

At necropsy, all rats utilized in this study were in good general health. No exposure-related gross lesions were noted during necropsy. Mild hydronephrosis was observed in the kidneys of one mid-concentration and one control male rat. Unilateral seminal vesicle atrophy was observed in one high- and one mid-concentration male rat. A 1-cm flat, tan area was observed on the dorsal surface of the median lobe of the liver on one control male rat.

Histopathology

Statistical analyses revealed a significant incidence of minimal pulmonary inflammation in the lungs of the high- ($p<0.05$) and the mid-concentration ($p<0.01$) male rats. This inflammation was determined to be either perivascular or interstitial (Table 13). Perivascular inflammation consisted of various combinations of mononuclear cells which multifocally formed cuffs around small veins and arterioles. Interstitial inflammation was often present in animals with perivascular inflammation and consisted of similar inflammatory cells extending out into the surrounding parenchyma, occasionally associated with type II cell hyperplasia, fibrosis, and intra-alveolar accumulations of foamy macrophages.

Lesions observed in the remaining tissues exhibited were common for rats during subchronic studies and did not occur at statistically significant higher incidence in any treatment group compared to the controls.

Reproductive Indices

The exposures showed no adverse effects on mating; The mating index was 100% for the high-, low-, and control groups mated. Mating occurred at 83% in the mid-concentration group (Table 14). The fertility index was 90% in the mid-concentration group and was 100% in high-, low-, and control groups. No significant exposure-related differences were noted in length of gestation, sex ratio, gestation index, or mean number of offspring per litter. During the 21-day lactation phase, mean pup weights were statistically significant lower between the high-concentration group and control group (Table 15 and Figures 3 and 4). Mid-concentration mean pup weights were only statistically different at LD 1. Pups in 6/12 high-concentration litters were found to have numerous cuts on the skin during the first week after parturition. These cuts were determined to be bites from the dams. Three mid-concentration litters and one low-concentration litter had pups displaying these marks. No injuries of this type were found on pups of control litters. Gross necropsy at weaning of all pups revealed no gross lesions or exernal abormalities.

Neurotransmitter Analysis

Statistically significant treatment-related increases in DA were detected in quadricyclane-exposed male and female rats in all brain regions analyzed. In male rats, the increase in DA concentration was greatest in the brain stem (Table 16), while in female rats, the largest increase of DA occurred in the cerebellum (Table 17). Elevated levels of the metabolites of DA were also found in brain regions of quadricyclane-exposed rats. HVA was elevated in the septum, thalamus, and cerebellum in both male and female treated animals and Dopac was increased in the septum of both male and female treated animals.

For male quadricyclane-exposed animals, the largest increase in 5-HT was found in the hypothalamus region of the brain. 5-HT levels were increased greatest in female exposed animals in the cerebral cortex and caudate nucleus. Statistically significant increases in 5-HT levels were observed in all nine brain regions of high-concentration males rats, (Table 18). Mid-concentration males had significant increases in 5-HT levels in all brain regions except for the cerebellum. Low-concentration male 5-HT levels were increased over control in five of the nine brain regions. Quadricyclane-exposed

female rats had significant ($p<0.01$) increases in 5-HT levels in all brain regions except the cerebral cortex (Table 19).

Increases in E levels were detected in the caudate nucleus, septum, and the cerebral cortex of male and female treated animals. No significant changes in neurotransmitter levels, in either male or female rats, were detected in any region of brain for NE, or for 5-HT's metabolite 5-HIAA.

Table 7. Analysis of Quadricyclane Concentrations Inhaled by Male and Female Rats During General Toxicity/Reproductive Screen (N=84)

Target Concentration, mg/L	0.10	0.025	0.01
Mean Measured Concentration, mg/L	0.101	0.025	0.01
Standard Deviation	0.011	0.001	<0.001
Lowest Daily Average, mg/L	0.093	0.023	0.009
Highest Daily Average, mg/L	0.108	0.026	0.011
Mean Temperature (°F)	73	74	74
Mean Relative Humidity (%)	58	55	57

Figure 1. Mean Body Weights of Male Rats Treated with Quadricyclane During the General Toxicity/Reproductive Screen

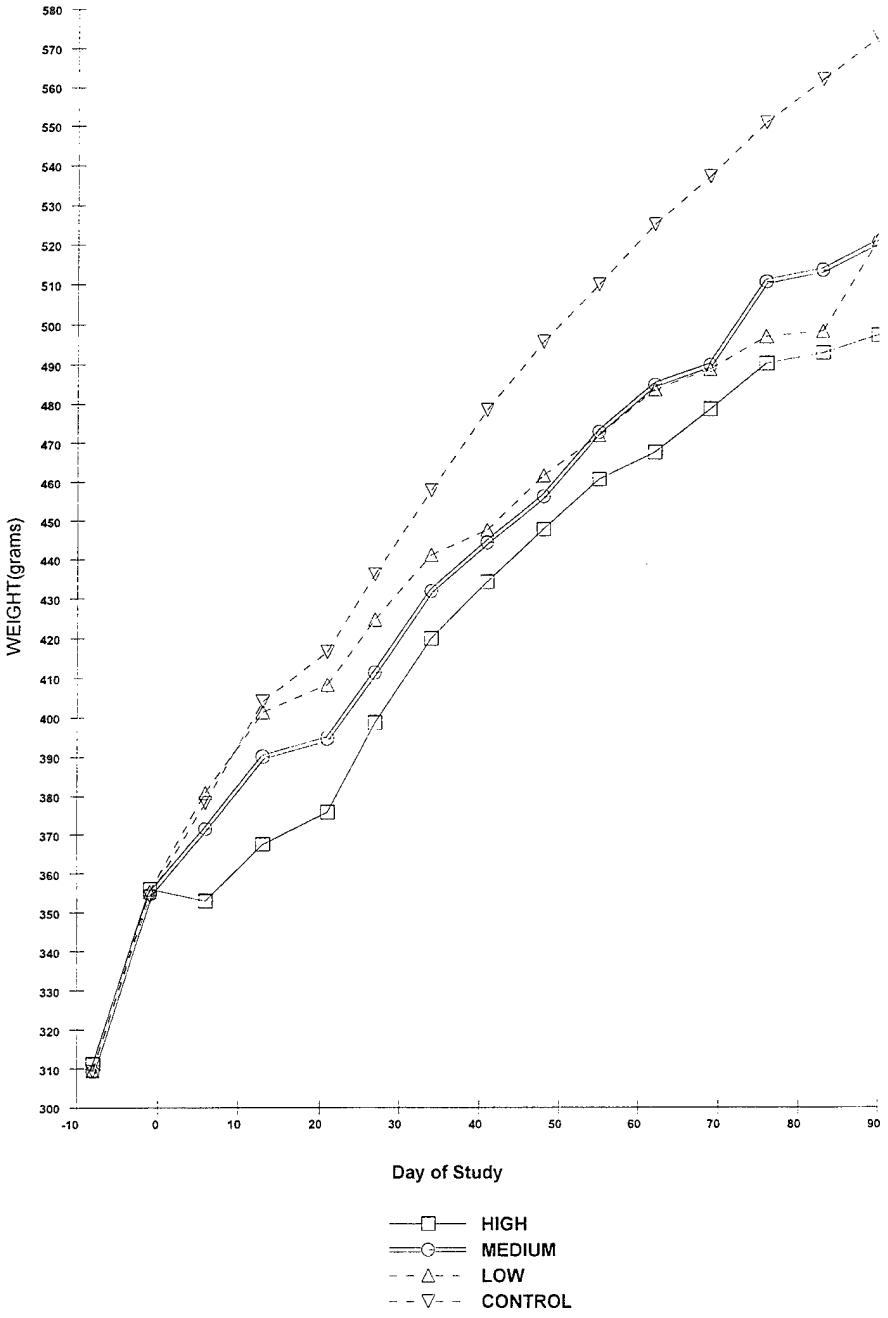
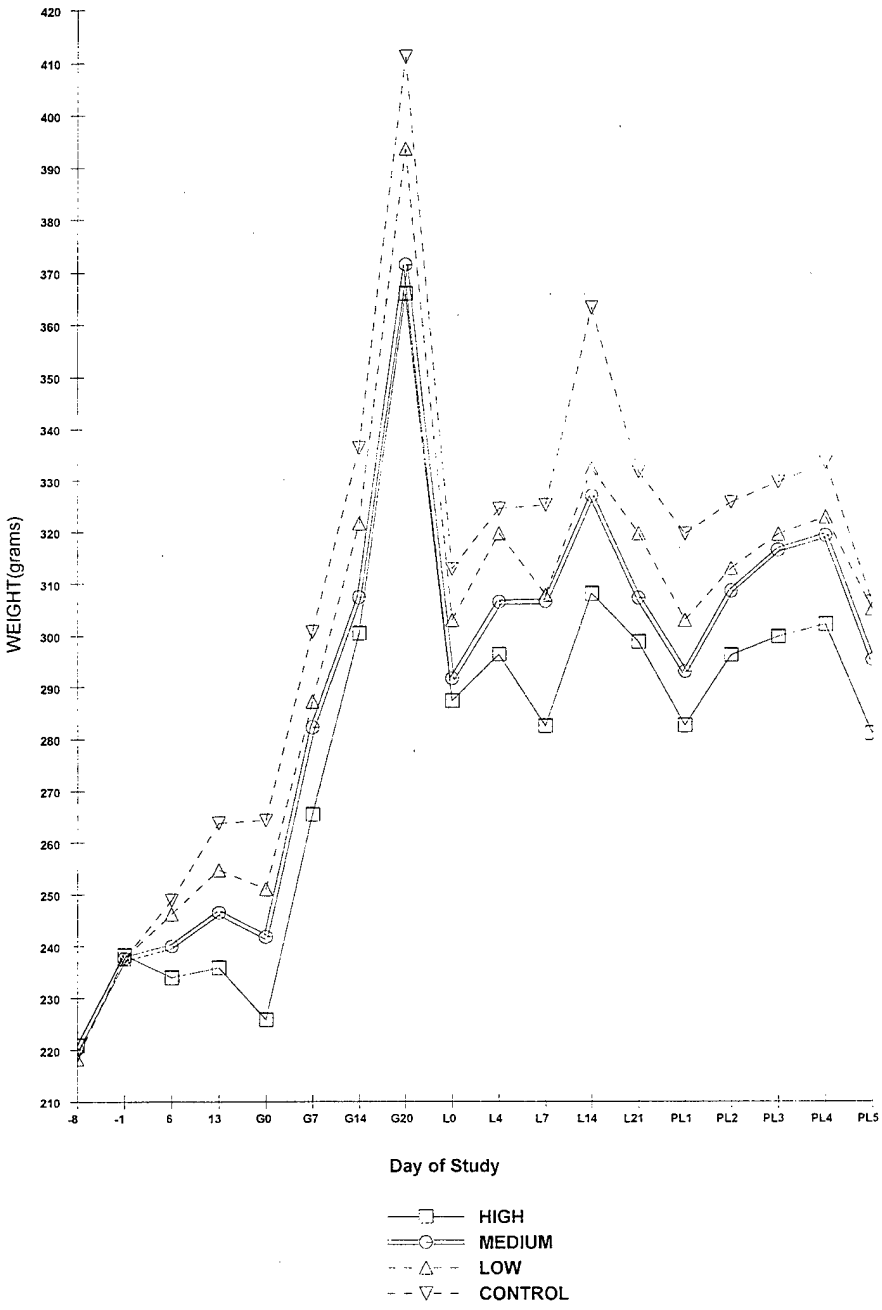


Figure 2. Mean Body Weights of Female Rats Treated with Quadricyclane During the General Toxicity/Reproductive Screen



Footnote:

G= Gestation
L= Lactation
PL= Post lactation

Table 8. Blood Hematology Values[a] of Male Rats Following Treatment with Quadricyclane During the General Toxicity/Reproductive Screen

	Control[b]	Low	Medium	High
WBC (10^3)	13.77 ± 0.99	12.32 ± 0.79	12.45 ± 0.78	11.86 ± 0.69
RBC (10^6)	8.94 ± 0.12	8.96 ± 0.21	8.75 ± 0.11	8.97 ± 0.17
HGB (g/dL)	15.49 ± 0.23	15.36 ± 0.29	15.26 ± 0.18	15.28 ± 0.16
HCT (%)	50.23 ± 0.74	50.00 ± 0.81	49.52 ± 0.56	49.42 ± 0.54
MCV (fL)	56.16 ± 0.43	55.87 ± 0.54	56.65 ± 0.57	55.27 ± 0.79
MCH (pg)	17.34 ± 0.16	17.19 ± 0.13	17.45 ± 0.22	17.08 ± 0.23
MCHC (g/dL)	30.86 ± 0.14	30.77 ± 0.19	30.78 ± 0.11	30.88 ± 0.12
Platelets (10^3)	1199 ± 21	1267 ± 47	1205 ± 25	1172 ± 36
Neutrophils (%)	10.56 ± 0.97	13.28 ± 1.07	14.88 ± 1.64	12.08 ± 1.25
Lymphocytes (%)	78.13 ± 2.07	78.28 ± 1.66	76.36 ± 2.76	75.38 ± 2.30
Monocytes (%)	9.60 ± 1.62	6.81 ± 0.76	7.28 ± 1.30	10.98 ± 1.17
Eosinophils (%)	1.30 ± 0.39	1.46± 0.27	1.33 ± 0.49	1.24 ± 0.19
Basophils (%)	0.42 ± 0.22	0.17 ± 0.08	0.15 ± 0.06	0.33 ± 0.11

[a]Mean ± SEM, N=12.
[b]N=11.

Table 9. Blood Hematology Values[a] of Female Rats Following Treatment with Quadricyclane During the General Toxicity/Reproductive Screen

	Control	Low	Medium	High
WBC (10^3)	10.08 ± 0.91	9.53 ± 0.79	9.35 ± 0.75	8.71 ± 0.49
RBC (10^6)	8.20 ± 0.11	8.22 ± 0.13	8.13 ± 0.15	8.08 ± 0.19
HGB (g/dL)	14.68 ± 0.23	14.71 ± 0.20	14.72 ± 0.27	14.49 ± 0.24
HCT (%)	46.16 ± 0.59	46.23 ± 0.66	46.71 ± 0.96	45.81 ± 0.88
MCV (fL)	56.32 ± 0.25	56.22 ± 0.34	57.39 ± 0.47	56.70 ± 0.30
MCH (pg)	17.93 ± 0.09	17.89 ± 0.10	18.08 ± 0.16	17.93 ± 0.15
MCHC (g/dL)	31.82 ± 0.16	31.83 ± 0.10	31.53 ± 0.13	31.63 ± 0.14
Platelets (10^3)	1229 ± 39	1231 ± 74	1131 ± 56	1191 ± 46
Neutrophils (%)	8.07 ± 1.04	9.13 ± 1.17	10.12 ± 0.86	7.31 ± 0.59
Lymphocytes (%)	82.02 ± 1.51	76.31 ± 2.58	76.90 ± 2.44	81.82 ± 1.97
Monocytes (%)	8.10 ± 0.90	11.83 ± 1.48	10.89 ± 1.80	9.15 ± 1.66
Eosinophils (%)	1.18 ± 0.14	1.54 ± 0.14	1.28 ± 0.15	1.13 ± 0.08
Basophils (%)	0.66 ± 0.19	1.20 ± 0.28	0.81 ± 0.27	0.65 ± 0.19

[a]Mean ± SEM, N=12.

Table 10. Mean Values[a] of Serum Chemistry Parameters for Male Rats Following Treatment with Quadricyclane During the General Toxicity/Reproductive Screen

	Control[b]	Low	Medium	High
Urea nitrogen (mg/kg)	13.82 ± 0.48	14.25 ± 0.84	13.42 ± 0.50	11.25 ± 0.25
Creatinine (mg/dL)	0.63 ± 0.02	0.59 ± 0.03	0.56 ± 0.02	0.54 ± 0.02[c]
Chloride (mmol/L)	98.91 ± 0.37	98.25 ± 0.41	98.25 ± 0.65	99.17 ± 0.42
Calcium (mg/dL)	11.68 ± 0.08	11.18 ± 0.07	11.45 ± 0.10	11.60 ± 0.14
Phosphorus (mg/dL)	1.40 ± 0.64	11.00 ± 0.70	11.08 ± 0.68	11.95 ± 1.35
Total protein (g/dL)	7.06 ± 0.04	7.04 ± 0.06	7.00 ± 0.09	7.06 ± 0.09
AST (IU/L)	104.7 ± 11.8	100.1 ± 6.4	85.92 ± 6.01	89.83 ± 9.93
ALT (IU/L)	50.09 ± 2.58	53.33 ± 3.24	48.17 ± 3.10	39.58 ± 1.52
Alkaline phosphatase (IU/L)	127.4 ± 8.4	161.3 ± 14.3[c]	131.4 ± 8.9	118.3 ± 8.1
Glucose (mg/dL)	202.3 ± 22.8	178.5 ± 15.6	182.2 ± 12.7	188.5 ± 20.4
Sodium (mmol/L)	146.8 ± 0.4	149.0 ± 0.5[c]	148.1 ± 0.6	148.9 ± 0.7[c]
Triglycerides (mg/dL)	188.4 ± 26.5	122.5 ± 13.5[c]	116.0 ± 11.1[c]	138.7 ± 8.6
Magnesium (mg/dL)	3.35 ± 0.15	3.52 ± 0.17	3.32 ± 0.15	3.30 ± 0.16
Potassium (mmol/L)	6.62 ± 0.17	5.38 ± 0.22[d]	5.89 ± 0.23[c]	5.42 ± 0.15[d]
Cholesterol (mg/dL)	66.55 ± 2.83	62.46 ± 2.28	67.60 ± 2.70	68.50 ± 2.00
Total bilirubin (mg/dL)	0.31 ± 0.03	0.33 ± 0.02	0.35 ± 0.04	0.34 ± 0.03
CO_2 (IU/L)	30.73 ± 0.89	34.00 ± 0.55[d]	33.25 ± 0.83	32.50 ± 1.00
Uric acid (mg/dL)	3.01 ± 0.32	2.17 ± 0.28	2.48 ± 0.26	2.43 ± 0.23
Albumin (g/dL)	3.75 ± 0.05	3.72 ± 0.05	3.66 ± 0.06	3.73 ± 0.08
Globulin (g/dL)	.30 ± 0.05	3.33 ± 0.06	3.32 ± 0.06	3.32 ± 0.04
Creatine kinase (IU/L)	65.18 ± 5.99	79.92 ± 12.44	66.42 ± 6.55	66.50 ± 11.03

[a]Mean ± SEM, N=12.
[b]N=11.
[c]Different from control, $p<0.05$.
[d]Different from control, $p<0.01$.

Table 11. Mean Values[a] of Serum Chemistry Parameters for Female Rats Following Treatment with Quadricyclane During the General Toxicity/Reproductive Screen

	Control	Low	Medium	High
Urea nitrogen (mg/kg)	14.58 ± 0.77	15.92 ± 0.78	15.42 ± 0.74	14.92 ± 0.69
Creatinine (mg/dL)	0.57 ± 0.01	0.53 ± 0.02	0.56 ± 0.01	0.52 ± 0.01
Chloride (mmol/L)	99.67 ± 0.57	99.75 ± 0.54	100.9 ± 0.6	100.1 ± 0.4
Calcium (mg/dL)	11.53 ± 0.16	11.50 ± 0.10	11.41 ± 0.07	11.39 ± 0.09
Phosphorus (mg/dL)	7.54 ± 0.38	7.51 ± 0.21	7.68 ± 0.22	7.44 ± 0.24
Total protein (g/dL)	7.76 ± 0.08	7.48 ± 0.08	7.41 ± 0.08[b]	7.42 ± 0.09[b]
AST (IU/L)	79.92 ± 8.82	71.50 ± 4.61	75.50 ± 5.56	65.08 ± 4.68
ALT (IU/L)	38.42 ± 1.95	34.58 ± 1.71	38.17 ± 2.95	34.67 ± 3.98
Alkaline phosphatase (IU/L)	81.58 ± 8.33	80.17 ± 5.61	73.83 ± 5.31	72.42 ± 5.54
Glucose (mg/dL)	188.9 ± 10.2	195.7 ± 10.1	193.7 ± 10.9	170.8 ± 10.1
Sodium (mmol/L)	146.9 ± 0.4	146.7 ± 0.3	147.2 ± 0.5	148.2 ± 0.5
Triglycerides (mg/dL)	145.7 ± 12.0	126.3 ± 22.8	103.0 ± 17.1	118.3 ± 11.8
Magnesium (mg/dL)	3.08 ± 0.08	3.21 ± 0.08	3.05 ± 0.09	2.97 ± 0.07
Potassium (mmol/L)	5.99 ± 0.17	5.58 ± 0.17	5.60 ± 0.17	5.25 ± 0.14[b]
Cholesterol (mg/dL)	71.33 ± 3.46	73.50 ± 3.53	68.00 ± 2.86	77.00 ± 4.21
Total bilirubin (mg/dL)	0.38 ± 0.02	0.38 ± 0.03	0.35 ± 0.02	0.35 ± 0.02
CO_2 (IU/L)	32.25 ± 0.57	32.75 ± 0.55	32.33 ± 0.61	34.42 ± 0.47
Uric acid (mg/dL)	2.33 ± 0.22	2.08 ± 0.18	1.90 ± 0.14	1.65 ± 0.19
Albumin (g/dL)	4.31 ± 0.09	4.07 ± 0.07	4.04 ± 0.07	4.15 ± 0.10
Globulin (g/dL)	3.47 ± 0.05	3.41 ± 0.05	3.37 ± 0.06	3.28 ± 0.05
Creatine kinase (IU/L)	43.10 ± 7.03	49.30 ± 4.90	52.87 ± 7.38	50.00 ± 5.08

[a]Mean ± SEM, N=12.
[b]Different from control, $p<0.05$.

Table 12. Absolute (g) and Relative (%) Organ Weights[a] of Rats Exposed to Quadricyclane During the General Toxicity/Reproductive Screen

Organ	Control	Low	Medium	High
Males				
Liver	16.71 ± 0.7	14.20 ± 0.5	14.34 ± 0.5	13.38 ± 0.5[c]
Ratio[b]	3.00 ± 0.1	2.84 ± 0.1	2.85 ±<0.1	2.80 ± 0.1
Testes	3.33 ± 0.8	3.42 ± 0.1	3.52 ± 0.1	3.53 ± 0.1
Ratio	0.60 ± 0.01	0.69 ± 0.02[d]	0.70 ± 0.02[c]	0.74 ± 0.02[c]
Body Wt.	556.3 ± 11.4	500.6 ± 14.7	501.6 ± 11.5	477.6 ± 9.0[c]
Females				
Liver	9.56 ± 0.3	8.65 ± 0.3	8.16 ± 0.2	7.85 ± 0.2[c]
Ratio	2.97 ± 0.1	2.82 ± 0.1	2.70 ± <0.1	2.75 ± 0.1
Body Wt.	321.1 ± 6.4	306.8 ± 5.4	302.0 ± 6.8	285.4 ± 5.4[c]

[a]Mean ± SEM, N=12.
[b]Organ weight/body weight x 100.
[c]Significantly different from control, $p<0.01$.
[d]Significantly different from control, $p<0.05$.

Table 13. Incidence Summary of Selected Microscopic Lesions of Male Rats Following Treatment with Quadricyclane During the General Toxicity/Reproductive Screen

Organ/Lesion	Control	Low	Medium	High
Lung (N)	11	12	12	12
Perivascular inflammation (N)	0	0	11[a]	6[b]
(severity)[c]	0.0	0.0	0.9	0.5
Interstitial inflammation (N)	0	0	8[a]	5[b]
(severity)[c]	0.0	0.0	0.8	0.7

[a]Statistically different from control at $p<0.01$.
[b]Statistically different from control at $p<0.05$.
[c]Mean grades of severity based on
0 = Normal; 1 = Minimal; 2 = Mild; 3 = Moderate;
4 = Marked; 5 = Severe.

Table 14. Litter Data for Rats Treated with Quadricyclane

Dose Level:	Control	Low	Medium	High
Number of Mated Pairs	12	12	12	12
Number of Copulated Pairs	12	12	10	12
Number of Dams with Pups Born	12	12	9	12
Number of Dams with Pups Alive	12	12	9	12
Mean Number of Pups per Litter	15.3	15.6	13.9	13.5
Average Length of Gestation (days)	22.5	22.5	22.3	22.4
Gestation Index (%)[a]	100.0	100.0	90.0	100.0
Live Birth Index (%)[b]	98.9	98.4	98.4	95.7
4-Day Survival Index (%)	99.5	98.4	98.4	95.7
7-Day Survival Index (%)	100.0	100.0	100.0	100.0
14-Day Survival Index (%)	100.0	100.0	100.0	100.0
21-Day Survival Index (%)	100.0	100.0	100.0	99.0
Lactation Index (%)	100.0	100.0	100.0	99.0

[a] Number of females with live litters

Number of females pregnant

[b] Number of live pups at birth

Total number of pups born

[c] Number of pups surviving 21 days

Number of pups surviving 4 days

Table 15. Mean Body Weights[a] of Male and Female Rat Pups

Day	Control	Low	Medium	High
		Male		
Day 1	7.10 ± 0.08	7.02 ± 0.94	6.55 ± 0.10[b]	6.45 ± 0.09[b]
N	83	90	64	71
Day 4	9.80 ± 0.17	9.90 ± 0.14	9.33 ± 0.16	9.06 ± 0.18[c]
N	83	90	64	67
Day 7	15.29 ± 0.33	15.35 ± 0.29	14.84 ± 0.29	13.21 ± 0.32[b]
N[d]	49	48	36	43
Day 14	28.60 ± 0.52	29.02 ± 0.44	27.21 ± 0.42	24.05 ± 0.46[b]
N	49	48	36	43
Day 21	46.69 ± 1.02	47.60 ± 0.89	44.42 ± 1.01	37.36 ± 0.84[b]
N	49	48	36	43
		Female		
Day 1	6.70 ± 0.74	6.54 ± 0.09	6.24 ± 0.10[c]	5.94 ± 0.08[b]
N	99	91	57	80
Day 4	9.27 ± 0.13	9.30 ± 0.14	9.21 ± 0.16	8.32 ± 0.18[b]
N	98	91	56	76
Day 7	14.44 ± 0.34	14.64 ± 0.31	14.30 ± 0.29	12.74 ± 0.33[b]
N[d]	47	48	36	53
Day 14	27.63 ± 0.53	27.86 ± 0.49	26.69 ± 0.40	23.70 ± 0.44[b]
N	47	48	36	53
Day 21	44.69 ± 0.99	44.53 ± 0.88	43.47 ± 0.99	36.69 ± 0.86[b]
N	47	48	36	51

[a]Mean ± SEM.

[b]Significantly different than Control at $p<0.01$.

[c]Significantly different than Control at $p<0.05$.

[d]Each litter culled to 4 male and 4 female, when possible, after Day 4 observations.

Table 16. Concentration[a] of the Neurotransmitter Dopamine in Nine Brain Regions of Male Rats Exposed to Quadricyclane

Brain Region	Control	Low	Medium	High
Septum	15.9 ± 0.5	16.8 ± 1.5	26.6 ± 1.6[c,d]	40.3 ± 1.9[b,c,d]
Hypothalamus	6.9 ± 0.3	18.8 ± 0.7[d]	24.0 ± 1.2[d]	44.7 ± 0.7[b,c,d]
Brain Stem	3.7 ± 0.2	9.9 ± 0.3[d]	18.8 ± 1.1[c,d]	29.7 ± 0.8[b,c,d]
Cerebral Cortex	9.2 ± 0.3	10.6 ± 0.5[d]	22.6 ± 0.7[c,d]	36.7 ± 2.0[b,c,d]
Caudate Nucleus	10.6 ± 0.5	14.0 ± 0.6	34.3 ± 1.1[c,d]	58.1 ± 0.8[b,c,d]
Frontal Cortex	7.7 ± 0.2	12.9 ± 0.6[d]	13.6 ± 0.9[c,d]	17.5 ± 0.2[b,c,d]
Cerebellum	8.9 ± 0.4	11.7 ± 0.5[d]	20.3 ± 0.8[d]	47.1 ± 2.2[c,d,e]
Thalamus	5.3 ± 0.3	8.8 ± 0.5[d]	29.1 ± 1.0[c,d]	35.6 ± 1.2[b,c,d]
Hippocampus	15.4 ± 0.6	22.3 ± 0.7[d]	30.5 ± 0.8[c,d]	42.7 ± 0.6[c,d]

[a]μg Dopamine/g wet tissue weight ± SEM.
[b]Significantly different than medium at $p<0.01$.
[c]Significantly different than low at $p<0.01$.
[d]Significantly different than control at $p<0.01$.
[e]Significantly different than medium at $p<0.05$.

Table 17. Concentration[a] of the Neurotransmitter Dopamine in Nine Brain Regions of Female Rats Exposed to Quadricyclane

Brain Region	Control	Low	Medium	High
Septum	15.1 ± 0.7	24.6 ± 0.3[d]	55.5 ± 1.3[c,d]	83.3 ± 1.7[b,c,d]
Hypothalamus	8.9 ± 0.4	14.4 ± 0.5[d]	22.8 ± 0.5[c,d]	34.8 ± 0.7[b,c,d]
Brain Stem	10.9 ± 0.9	15.3 ± 0.4[d]	23.2 ±1.0[c,d]	32.6 ± 0.6[b,c,d]
Cerebral Cortex	15.1 ± 0.3	20.7 ± 0.6	23.8 ± 1.2[d]	39.3 ± 1.6[b,c,d]
Caudate Nucleus	11.0 ± 0.7	31.8 ± 0.7[d]	55.1 ± 0.9[c,d]	79.8 ± 2.4[b,c,d]
Frontal Cortex	9.6 ± 0.6	16.7 ± 0.5[d]	23.9 ± 0.8[c,d]	36.0 ± 1.0[b,c,d]
Cerebellum	5.4 ± 0.2	22.7 ± 0.3	33.2 ± 1.4[c,d]	69.5 ± 1.9[b,c,d]
Thalamus	10.1 ± 0.3	18.7 ± 0.7[d]	29.0 ± 0.7[c,d]	36.8 ± 1.7[c,d]
Hippocampus	17.0 ± 0.3	24.0 ± 0.6[d]	36.7 ± 0.9[c,d]	47.0 ± 1.2[c,d,e]

[a]μg Dopamine/g wet tissue weight ± SEM.
[b]Significantly different than medium at $p<0.01$.
[c]Significantly different than low at $p<0.01$.
[d]Significantly different than control at <0.01.
[e]Significantly different than medium at <0.05.

Table 18. Concentration[a] of the Neurotransmitter 5-Hydroxytryptamine in Nine Brain Regions of Male Rats Exposed to Quadricyclane

Brain Region	Control	Low	Medium	High
Septum	1.0 ± 0.1	1.0 ± 0.1	2.0 ± 0.1[c,d]	4.3 ± 0.2[b,c,d]
Hypothalamus	0.2 ±<0.1	0.5 ±<0.1[d]	0.5 ±<0.1[d]	1.2 ±<0.1[b,c,d]
Brain Stem	0.6 ±<0.1	1.1 ± 0.1[d]	0.9 ±<0.1[d]	1.2 ± 0.1[d]
Cerebral Cortex	0.9 ± 0.1	1.1 ± 0.1	0.5 ±<0.1[c,d]	1.1 ±<0.1[b]
Caudate Nucleus	0.4 ±<0.1	0.3 ±<0.1[d]	1.1 ±<0.1[c,d]	1.1 ± 0.1[c,d]
Frontal Cortex	0.7 ±<0.1	0.5 ±<0.1[e]	0.8 ±<0.1[c]	0.9 ±<0.1[c]
Cerebellum	0.3 ±<0.1	0.2 ±<0.1	0.3 ±<0.1	0.9 ± 0.1[b,c,d]
Thalamus	0.4 ±<0.1	1.0 ± 0.1[d]	1.2 ± 0.1[d]	1.3 ± 0.1[d]
Hippocampus	0.2 ±<0.1	0.2 ±<0.1	1.0 ±<0.1[c,d]	1.0 ± 0.1[c,d]

[a]µg 5-Hydroxytryptamine/g wet tissue weight ± SEM.
[b]Significantly different than medium at $p<0.01$.
[c]Significantly different than low at $p<0.01$.
[d]Significantly different than control at $p<0.01$.
[e]Significantly different than control at $p<0.05$.

Table 19. Concentration[a] of the Neurotransmitter 5-Hydroxytryptamine in Nine Brain Regions of Female Rats Exposed to Quadricyclane for

Brain Region	Control	Low	Medium	High
Septum	0.8 ± 0.1	1.0 ±<0.1	2.0 ±<0.1[b,c]	2.0 ± 0.1[b,c]
Hypothalamus	0.5 ±<0.1	1.2 ±<0.1[c]	1.3 ±<0.1[c]	1.5 ± 0.1[b,c]
Brain Stem	0.4 ±<0.1	0.5 ±<0.1[c]	1.1 ± 0.1[b,c]	1.3 ±<0.1[b,c]
Cerebral Cortex	1.1 ± 0.2	1.0 ±<0.1	1.0 ±<0.1	1.1 ± 0.1
Caudate Nucleus	0.3 ±<0.1	1.0 ±<0.1[c]	1.3 ±<0.1[c]	1.3 ± 0.1[c]
Frontal Cortex	0.5 ±<0.1	1.1 ±<0.1[c]	1.3 ± 0.1[c]	1.2 ±<0.1[c]
Cerebellum	0.4 ±<0.1	1.0 ±<0.1[c]	1.1 ±<0.1[c]	1.2 ± 0.1[c]
Thalamus	0.5 ±<0.1	1.0 ±<0.1[c]	1.2 ±< 0.1[c]	1.2 ± 0.1[c]
Hippocampus	0.4 ±<0.1	0.9 ±<0.1[c]	1.0 ± 0.1[c]	1.0 ± 0.1[c]

[a]µg 5-Hydroxytryptamine/g wet tissue weight ± SEM.
[b]Significantly different than low at $p<0.01$.
[c]Significantly different than control at $p<0.01$.

Figure 3. Mean Body Weights of Male Pups During 21-Day Lactation Phase from Quadricyclane-Exposed Rats

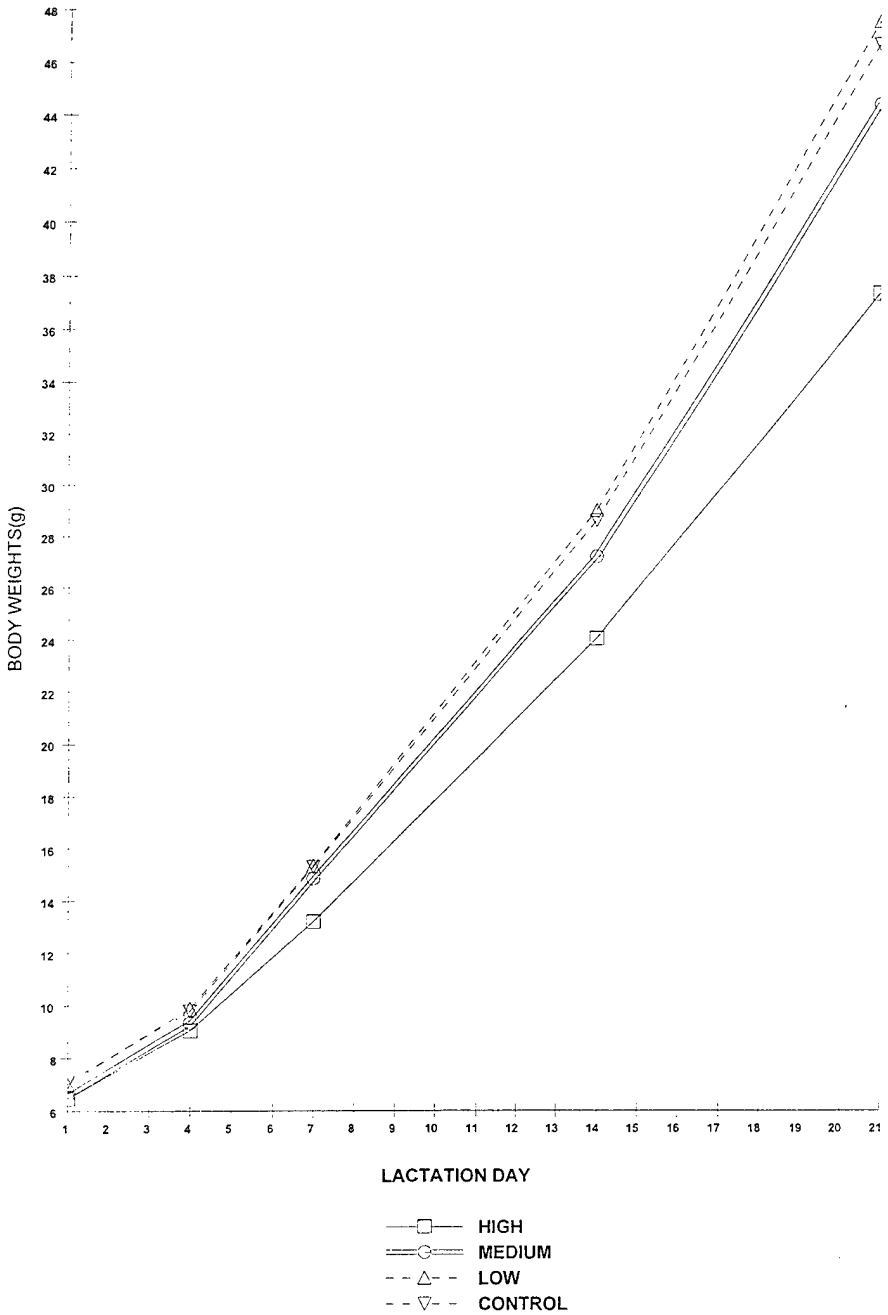
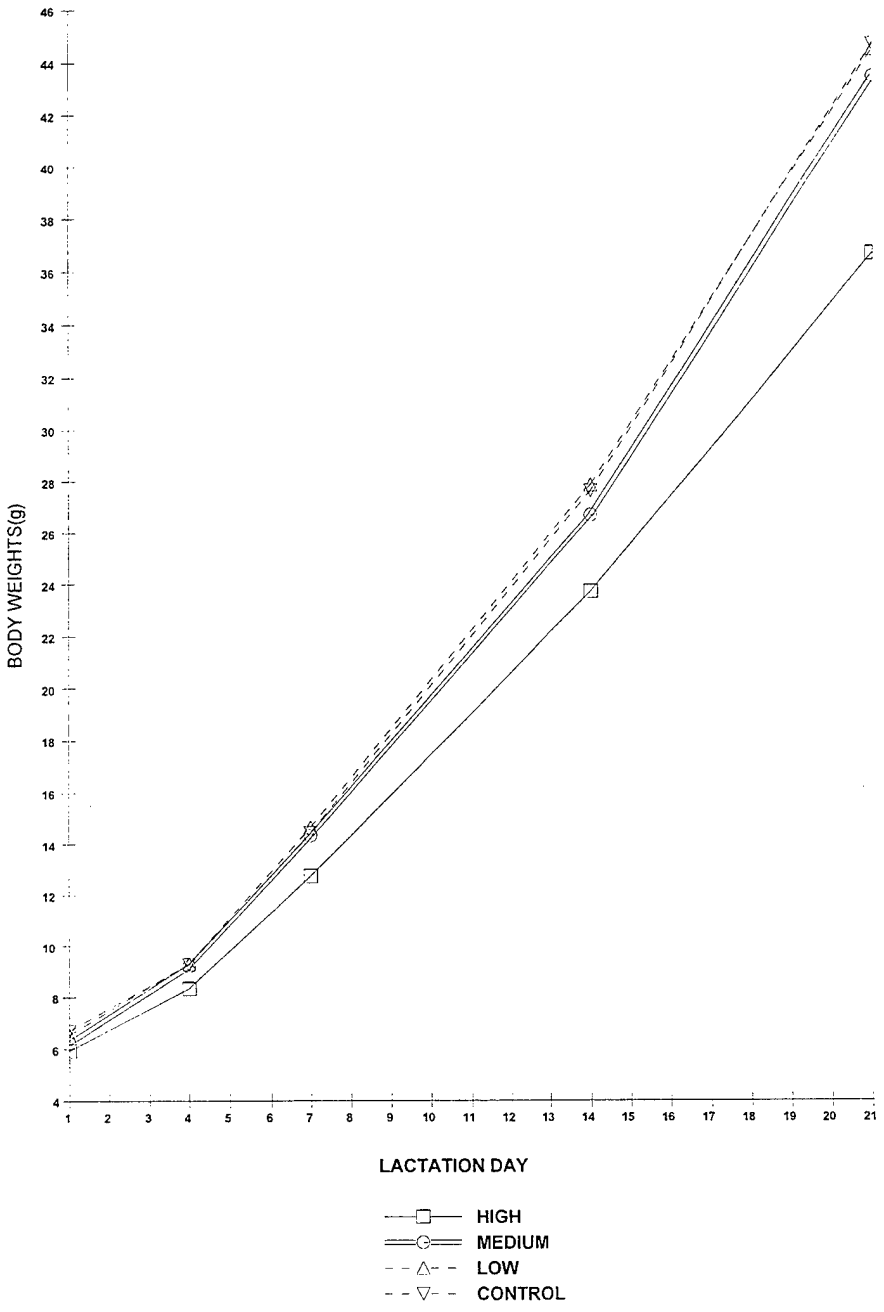


Figure 4. Mean Body Weights of Female Pups During 21-Day Lactation Phase from Quadricyclane-Exposed Rats



DISCUSSION

Inhalation of quadricyclane at the limit test concentration resulted in total mortality. The lack of delayed deaths (none after the first day posttreatment) and evidence of pulmonary congestion at necropsy suggest that death was caused by acute irritation of the respiratory system. Repeat exposure of male and female rats to quadricyclane vapor at concentrations of 0.25, 0.075, 0.025, and 0.0 mg/L resulted in no mortality. Animals in the high-concentration group became progressively aggressive during the course of the two-week study. Hyperethesia was also apparent in these animals. The solid white gelatinous plugs found in the excreta pans during the subchronic study were histopathologically determined to consist of a normal proteinaceous coagulum, and were not considered to be biologically significant. Treatment-related decreases in body weights were observed over the two-week exposure period. The only organ-to-body weight difference noted was for the brain in all exposed female rats and the high-concentration male rats. In general, the brain, does not increase or decrease in size with body weight changes and, therefore the absolute weight, which was not different from controls, is the better indicator of effect. Isolated differences in serum creatinine, alanine aminotransaminase, and calcium were noted only in high-concentration male rats, but were not considered biologically significant.

Inhalation exposure of male and female rats to quadricyclane vapors at concentrations of 0.10, 0.025, 0.01, and 0.0 mg/L produced no adverse effects on reproductive performance or litter parameters. Treatment-related decreases in mean body weight were noted in both male and female rats. High-concentration animals began to demonstrate hyperesthesia during the second week of exposure, and these animals became more aggressive as the study progressed. Mid-concentration female rats became aggressive and sensitive to touch only during the gestation period. The low-concentration animals did not demonstrate this behavior. The aggressive behavior of these animals is believed to be the direct cause of the bite marks noted in the pups, in which the incidence of bite marks also occurred in a treatment-related manner. Solid white gelatinous plugs were again found in the excreta pans of all male rats. These plugs were determined to be the same as those found during the subchronic exposure, and therefore were not determined to be biologically significant. At necropsy, all animals were in good general condition. No exposure-related gross lesions were noted at necropsy. Clinical pathology data revealed no significant alterations in hematology values in any group of rats. Isolated significant differences in serum chemistry were noted in male and female rats. The decrease in potassium was the only consistent finding in both sexes of the high-concentration group. This may be related to the significant body weight loss in these animals. A decrease in food consumption may also have been a contributing factor, but food intake was not measured during the study.

The only statistically significant histologic finding in rats following quadricyclane exposure was minimal pulmonary inflammation observed in 6of 12 high- and 11of 12 mid-concentration male rats. Similar lesions were not observed in the lungs of female rats. This type of lesion most likely represents an immunologic response and is compatible with lesions described in rats in the aftermath of a viral respiratory infection (Brownstein, 1985). The possibility that this lesion was a male rat-specific inflammatory reaction to inhalation of quadricyclane seems unlikely since the incidence did not occur in a concentration-related manner. The lesion was not thought to be biologically significant as the severity of the lung lesions was never more than mild, and no clinical signs of respiratory distress were reported in affected animals during the in-life portion of the study.

The most significant finding from the neurotransmitter analyses of brain tissue from quadricyclane-exposed male and female rats was an increase in dopamine levels in all brain regions. An increase in dopamine level was detected even in the low-concentration animals which did not display hyperesthesia or aggressive behavior. Antipsychotic drugs of phenothiazines and butrophenones class

(major tranquilizers) act on dopaminergic pathways and have produced calming effects in aggressive individuals and in experimental animal studies (Cooper et al.,1991). There is no direct experimental evidence that an excess of dopamine-dependent neuron activity or an elevation of dopamine levels at central nervous synapses causes behavioral alterations in animals or in humans. The role of 5-HT in aggressive behavior has been documented (Brown and Linnoila, 1990; Brunner et al., 1993). In a report by Saudou et al. (1994), mutant mice lacking the 5-HT_{1B} receptor were generated by homologous recombination. When confronted with an intruder, mutant mice attacked the intruder faster and more intensely than did the wild-type mice. Pharmacological studies with weak specific agonists have suggested that activation of 5-HT_{1B} receptors may lead to increased anxiety, locomotion, and increased aggressive behavior (Wilkinson and Dourish, 1991). Alterations in brain 5-HT levels in male and female quadricyclane-exposed rats may be one of the reasons for the aggressive behavior observed during the studies. Hyperesthesia observed in quadricyclane-exposed rats may be due to some alterations in the filtering mechanism in the reticular formation where afferent impulses from different sensory modalities are processed before they reach the sensory cortex in the cerebrum (Spence, 1992). Increased sensitivity to environmental stimuli such as sound, light, temperature, and touch can make an animal highly irritable, which may progress into aggressive behavior.

The results of this study indicate effects were seen at all of the exposure concentrations. The effects in female rats at the low-concentration level were limited to neurotransmitter changes. The increase in neurotransmitter levels is of unknown significance and may represent a reversible, physiological response to quadricyclane. The low-concentration level of 0.01 mg/L quadricyclane probably represents a NOAEL based on the female rat data and a lack of consistent dose-response data for male rats. In addition, the body weight depression for male rats in the low-concentration group was less than 10%.

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APPENDIX A

MEAN BODY WEIGHTS[a] OF MALE RATS EXPOSED TO QUADRICYCLANE FOR 90 DAYS

Day	Control[b]	Low	Medium	High
-8	309 ± 4.3	309 ± 3.9	309 ± 4.1	311 ± 4.1
-1	354 ± 5.2	355 ± 4.5	355 ± 5.5	356 ± 5.7
6	378 ± 6.1	381 ± 5.8	371 ± 6.4	353 ± 4.8[c]
13	404 ± 6.5	401 ± 5.1	390 ± 6.9	367 ± 5.1[d]
21	417 ± 6.5	408 ± 4.6	395 ± 8.4	376 ± 6.3
27	436 ± 7.5	425 ± 8.1	411 ± 8.7	399 ± 7.0[d]
34	458 ± 7.2	441 ± 7.1	432 ± 9.5[c]	420 ± 7.2[d]
41	479 ± 8.6	448 ± 7.6[c]	444 ± 9.6[d]	434 ± 7.3[d]
48	496 ± 8.2	462 ± 7.2[c]	456 ± 8.9[c]	448 ± 7.6[d]
55	510 ± 9.0	472 ± 10.0[d]	473 ± 8.5[d]	461 ± 8.3[d]
62	525 ± 8.9	484 ± 10.7[d]	485 ± 8.7[c]	468 ± 8.4[d]
69	537 ± 9.5	489 ± 14.1[d]	490 ± 10.0[c]	479 ± 8.3[d]
76	551 ± 10.3	497 ± 17.6[c]	511 ± 10.6	490 ± 8.9[d]
83	562 ± 10.8	499 ± 19.9[d]	514 ± 10.7[c]	493 ± 9.6[d]
90	573 ± 12.2	521 ± 13.3[c]	521 ± 11.9[c]	498 ± 9.9[d]

[a]Mean ± SEM, N=12.
[b]N=11.
[c]Significantly different than control at $p<0.05$.
[d]Significantly different than control at $p<0.01$.

APPENDIX B

BODY WEIGHTS[a] OF FEMALE RATS EXPOSED TO QUADRICYCLANE FOR 90 DAYS

Day	Control	Low	Medium	High
-8	219 ± 2.9	218 ± 3.2	220 ± 3.3	221 ± 3.1
-1	237 ± 3.3	238 ± 3.8	237 ± 3.6	238 ± 4.0
6	249 ± 4.0	246 ± 3.6	240 ± 4.6	234 ± 3.9
13	264 ± 4.3	255 ± 3.8	246 ± 4.6[c]	236 ± 4.8[b]
G0	264 ± 4.7	251 ± 4.3	242 ± 5.8[bd]	226 ± 4.5[b]
G7	301 ± 4.1	287 ± 4.7	282 ± 6.2[cd]	265 ± 4.8[b]
G14	336 ± 5.3	322 ± 5.6	307 ± 5.4[bd]	301 ± 5.6[b]
G20	411 ± 6.3	394 ± 7.0	372 ± 7.3[bd]	366 ± 8.5[b]
L0	313 ± 5.4	303 ± 6.3	292 ± 6.1[cd]	287 ± 4.8[b]
L4	324 ± 9.0	320 ± 5.7	306 ± 6.1[d]	296 ± 5.5[c]
L7	325 ± 5.4	308 ± 9.7	307 ± 6.8[d]	283 ± 6.3[b]
L14	363 ± 17.2	332 ± 7.3	327 ± 3.2[d]	308 ± 6.2[b]
L21	332 ± 5.8	320 ± 5.7	307 ± 4.3[cd]	299 ± 5.6[b]
PL1	320 ± 5.5[e]	303 ± 4.7	293 ± 4.6[b]	283 ± 5.3[b]
PL2	326 ± 5.6	313 ± 5.2	309 ± 6.0	296 ± 5.6[b]
PL3	330 ± 6.2	320 ± 6.1	317 ± 7.5	300 ± 6.4[b]
PL4	333 ± 5.6	323 ± 6.3	319 ± 7.8	302 ± 6.1[b]
PL5	306 ± 3.8[h]	305 ± 11.7[g]	295 ± 14.3[g]	281 ± 6.5[f]

[a]Mean ± SEM, N=12.
[b]Significantly different than control at $p<0.01$.
[c]Significantly different than control at $p<0.05$.
[d]N=9.
[e]N=11.
[f]N=7.
[g]N=5.
[h]N=9.